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Toxicological Effect of *Azadirachta Indica*

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Abstract: Toxicology is the study of symptoms, mechanism, treatments and detections of poisoning, especially the poisoning of people. All beneficial plants are also somewhat toxicological in nature. Neem is regarded as a promising tree species which can be utilized in variety ways to benefit agricultural communities throughout the world. Neem is natural source of insecticides, pesticides and agrochemicals and also used as bio-control agent to control many plant disease. Sometimes they produce a toxic effect on flora and fauna causes adverse effect on living organism. But their toxicological character are beneficial in way of killing harmful insect & pest, which feeds on theirs associated crops and this insect are also a part of ecosystem (food chain and food web), so the loss of insect causes biodiversity will gets jeopardized.

Keywords: Agrochemical, biodiversity, ecosystem, food chain.

Introduction

Toxicology (from Greek words toxicos "Poisonous" and logos) is a branch of biology, chemistry and medicine (more specifically pharmacology) concerned with the study of the adverse effects of chemicals on living organisms (Schrage, 2006). *Azadirachta indica* is a tree in the family Meliaceae as is native to India, Pakistan and Bangladesh growing tropical and subtropical regions. Neem shows medicinal in properties that cause also regarded as "the village pharmacy", "doctor tree" and "free tree of India". It has reputed value for its herbal medicine, spermicidal and hence treated as perfect, complete and imperishable gift in the nature (Jhariya et al., 2013). India stands first in neem seed production and about 4, 42,300 tons of seeds are produced annually yielding 88,400 tons of neem oil and 3, 53,800 tons of neem cake. With beneficial effect sometimes it has also bad effect on living organism. With banning of broad spectrum, toxic insecticides, such as DDT, the use of neem in crop protection has been increasing. Although a lot of work on pharmacological activity of neem extracts has been done, not much toxicological evaluation has been under taken. It is reported that leaves of neem because toxic effects on sheep (Ali and Salih, 1982) goats and guinea pigs (Ali, 1987). A dose higher than leading to death in guinea pigs. However, 200 mg/kg in the same route was found to be non-toxic to rabbits (Thompson and Anderson, 1978).

Composition

Neem seed oil composition: The neem oil contains acid like,

1. Oleic acid – 61.9%
2. Palmitic acid – 14.9%
3. Stearic acid – 14.4%
4. Linolic acid – 7.5%
5. Arachidic acid – 1.3%

The neem seed oil also contains Glycerides likes,

1. Fully saturated Glycerides – 0.6%
2. Tri-unsaturated Glycerides – 22%
3. Stearodiolein – 34%
4. Palmito-diolein – 26.0%
5. Oleopalmitostearin – 12%
6. Oleodipalmitin – 5%

The neem oil also contains 1.2- 1.6% Nimbidin, 0.1% Nimbin and 0.01% Nimbinin and 0.2- 1.0% Azadirachtin. Nimbidin cause bitterness of oil, contain sulphur. On hydrolysis nimbidin change into nimbidinic acid.

Neem leaf composition:

1. Crude protein – 12.40- 18.27%
2. Crude fibre – 11.40- 23.08%
3. N free extract – 43.32- 66.60%
4. Ether extract – 2.27- 6.24%
5. Total ash – 7.75- 18.37
6. Calcium – 0.89- 3.96%
7. Phosphorus – 0.10- 0.30%

Gum and resins composition:

Gum which exudes from the bark occurs in the form of clear, bright amber colored.

1. Ash- 3%
2. Water – 13- 15%
3. Sugar – 6.5%
4. Galactans – 12%
5. Pentosans – 26%
6. Albumins and oxidase
7. Albuminous and gummy matter – 6.5%

Neem cake:

Neem cake is the by-product of neem seeds and average nutrient content are,

1. Nitrogen – 5.2%
2. P_2O_5 – 1.0%
3. K_2O – 1.4%

Neem cake is both organic nitrogen manure and nitrification inhibitor in nature.

Types of Toxicity

1. Acute toxicity
2. Sub-acute toxicity

1. Acute toxicity

It describes the adverse effect of a substance that result either from a single exposure ("The MSDS Hyper Glossary, 2006) or from multiple exposures in short space of time (usually less than 24 hours). To be described as acute toxicity, the adverse effects should occur within 14 days of the administration of substance.

Acute oral toxicity

Ethanol neem containing 3000 ppm azadirachtin (± 10%) is registered with the Environmental Protection Agency (EPA), USA. The data submitted on acute oral toxicity in rats showed no negative effect upto a dose of 5ml/kg (National academy Press, Washington D.C., 1992). In another study, methanolic leaf and bark extracts showed an oral LD_{50} (Lethal dose, 50%) of about 13g/kg in acute toxicity studies on mice's. Animal showed general signs of ill health and discomfort, gastro-intestinal spasm, apathy, refusal of water and feed and hypothermia. Mice died under terminal convulsions. No gross microscopic lesion was found on autopsy (Okpanyi and Ezeukwv, 1981). An extensive acute toxicity study in rats and rabbits using neem seed oil gave rat 24hr LD_{50} of 14 and 24 ml/kg respectively. Target organs of toxic effects were the central nervous system and lungs (Gandhi et al., 1988). But methanol soluble and insoluble fractions, from an aqueous leaf extract were not toxic within 24hr at an oral dose of 200mg/kg in mice (Singh et al., 1987).

Acute dermal toxicity

LC_{50} (Lethal conc., 50%) of Morgosan-O registered by EPA, USA is more than 2ml/kg in albino rabbits (National Academy Press, Washington D.C., 1992).

Primary skin irritation

An ethanol extract of the seeds when injected intradermally into the shaved skin of guinea pigs showed no significant skin sensitive reaction (Gupta and Bhaid, 1981). Albino rabbits were treated with Margosan-O in patches on shaved areas and on abraded areas. The results showed low to moderate primary irritation to the shaved area patch and high to moderate irritation into abraded area (National Academy Press, Washington D.C., 1992).

Eye irritation

When 0.1 mg of an ethanol extract of neem seed was instilled into the eyes of male albino New Zealand rabbits, no reaction was observed (Jeter, 1980). Solution of sodium nimbinate (1 to 5%) instilled into the eyes failed to causes any eye irritation or changes in papillary size (Gaitonde and Sheth, 1958). Ethanol extract of seed instilled into rabbit's eye also failed in causing any irritation (Gupta and Bhaid, 1981). EPA registered Morgoson-O when administered to one washed and one unwashed eye of albino rabbits over 7 days, showed minimal irritation in both the eyes (National Academy Press, Washington, D.C., 1992). So all the above experiment is done on the rats, rabbits and guinea pigs and they showed their response.

2. Sub-acute toxicity

Sub acute oral toxicity with nimbidin was evaluated in albino rats by single oral administration in albino rats by single oral administration of 25, 50 or 100mg/kg of 6 weeks (Pillai and Santhakumari, 1984). Progressive decrease in body weight, pulse and respiratory rates along with diarrhea were noted in guinea pigs (Ali, 1987). Similarly general weakness, decreased heart and respiratory rates, diarrhea without any hematological changes were observed in Nubian goats of green or dried neem leaves. Necropsy showed areas of hemorrhagic erosion, congestion, and degeneration of liver, kidneys, lungs, duodenum, brain and somniferous tubules (Ali and Salih, 1982). Oral doses upto 100mg/kg for 6 weeks in rats and 20mg/kg for 28 days in dogs did not cause any toxicity as evaluated by possible pathological changes in internal organs at necropsy (Pillai and Santhakumari, 1984).

Effects on Avian species

The water extract of neem berries when tested in poultry birds and closely observed for 7 days showed signs of toxicity like sluggish movement, drooping head, cyanosed comb etc. sixty percent of birds died due to fragile liver undergoing degenerative changes with focal congestions, retention of bile in gall bladder and congestion of kidneys with localized haemorrhages. This gives impression of hepatic and nephrite toxicity including cardiovascular involvement (Singh et al., 1985). Trial feeding of neem seeds to starter chicken caused severe hepatitis with necrotic patches, mild to severe nephritis with congestion and slight inflammation in intestine (Verma, 1974). However, there is likelihood of contamination of the seed with aflatoxin.

Toxicity to aquatic species

Neem extracts are also harmful to the aquatic species on a certain level and their concentration 0.005% aqueous emulsion the neem extract was non-toxic to insectivore's fish, Gambusia species, 100% of them were killed in 24hr by a concentration of 0.04%. This concentration killed 80% of tadpoles in 24hr and 100% in 2 days. However, this extracts is non toxic

to tadpoles at 0.01% concentration (Attri and Raviprasad, 1980).

Effects on soil and soil micro-organism

Neem is an environmentally friendly insecticide, so it is often used in high concentrations and this can lead to a heavy load of neem in soil. High neem soil concentrations can cause chronic toxicity to non-target organisms such as crustaceans (Daphnia magna and Hyalella azteca) through leaching from soil into waterways. Neem has also been shown to induce genotoxicity in rodents and fish. These effects raise concern over the safe use of neem as a pesticide in agricultural practices. Although earthworms are often used in terrestrial ecotoxicity evaluation, there is little information available on the effects of neem on earthworm immune competent cells and histology of the epidermis, skin, body wall, and intestinal lining. In earthworms, coelomocytes are the circulating leukocytes present in the coelomic cavity and play an important role in immune defense. They have been used to study the effect of genotoxicants such as nickel (Ni) and cadmium (Cd) on earthworms. The genotoxicity of neem to earthworm coelomocytes has not been reported. Earthworms are resilient organisms and can live in soil containing significant concentrations of chemicals, including some persistent insecticides. Ecologically, this is relevant, because several species of birds and mammals feed on earthworms, and therefore, any chemical accumulation can potentially lead to biomagnifications.

Effect of azadirachtin on insect

Azadirachtin is the most prominent constituent of a series of limonoides (tetranortriterpenoids) present in the seed kernels of neem. The azadirachtin affect growth, development, behavior, reproduction and metamorphosis in diverse insect taxa. Different sites have been identified as targets for azadirachtin, and it has generally been accepted that behavioral effects are through chemoreceptor mechanism, and growth related effects are due to interference with the neuroendocrine control of moulting and ecdysis. It is well known that generally inhibition of feeding behavior in insects result from either blockage of the input from phagostimulatory receptors or from deterrent cells or both (Chapman, 1974; Dethier, 1982). Neurophysiologic effect of azadirachtin on feeding response in several polyphagus and oligophagous lepidopteron larvae have revealed differences and complexities in chemoreceptor responses both between different chemoreceptor's in the same insect and between species (Simmonds and Blaney, 1984). The azadirachtin prevents apolysis and ecdysis, induce pharate mortality, or sometimes induces permanent larvae. These effects are dose-and-time dependent and relative to the mode of application (Koul et al., 1987). Prothoracic glands as the target site of azadirachtin action. Azadirachtin also reduces juvenile hormone (JH) titres in insects. It has direct effects on a whole variety of tissues and organs, which suggest more than one site of action: azadirachtin treatment includes lack of larval crochets (Haasler, 1984), lack of differentiation of tissues such as ommatidia of eyes at larval pupal moult (Schluter, 1985), differentiation of imaginable discs (Schluter, 1987), occurrences of block patches on the cuticle and cuticular melanization resulting in characteristic block spots is often seen azadirachtin treated insects (Malczewska et al., 1988). Azadirachtin seems to effect also on muscles, insect gut, central nervous system, immune system etc that cause death of insects.

Effect on humans

Neem seed oil intoxication has been reported. It produced occasional diarrhea, nausea and general discomfort (Chopra et al., 1965). Injection of sodium nimbidinate at 1g and feed in dose of 7g to human beings did not produce any local or general side effects (Bhide et al., 1958). In Malaysia, 13 infants suffered from cute poisoning after ingestion of 5ml of neem seed oil for minor ailments. They developed symptoms like vomiting, drowsiness, metabolic acidosis, polymorph nuclear leukocytosis and encephalopathy (Sinniah and Baskaran, 1981). These systems are akin to the Reyes syndrome. Children are badly affected by neem oil after their ingestion that causes vomiting, drowsiness, respiratory difficulty, seizures after 1.5hr of last dose. Liver became enlarged and child died after 12 days of ingestion of oil (Sinniah et al., 1982). These findings indicates that the oil may be involved in the etiology of Reyes syndrome which in turn may be caused by a synergistic effect of aflatoxin and other toxic components present in the oil (Sinniah et al., 1983). Also effects on the physiological and metabolic process of the human being.

Some ecological implications of neem insectide disturbances to zooplankton communities in forest pond enclosures:

A neem based insecticide, Neemix 4.5, was applied to forest pond enclosures at conc. of 10, 17 and 28 microgram l (-1) azadirachtin (the active ingredient). At this test conc., significant, conc. Dependent reductions in numbers of adult copepods were observed, but immature copepods and cladoceran populations were unaffected. There was no evidence of recovery of adult copepods within the sampling season (May to October). The ecological significance of this disturbance to the Zooplankton community was examined by determining biomass as a measure of food availability for higher predators, plankton community respiration, dissolved oxygen (DO) conc., and conductivity as functional indicators of ecosystem stress and Zooplankton food web stability as a measure of effects on trophic structures. The selective removal or reduction of

adult copepods was sufficient to measurably reduce total Zooplankton biomass for several weeks mid-season. During the period of maximal impact (about 4-9 weeks after the applications), total plankton community respiration was significantly reduced, and conc. Dependent increases in dissolved O_2 and decrease in conductivity among treated enclosures. The reductions in adult copepods resulted in negative effects on Zooplankton food web stability through eliminations of a trophic link and reduced interactions and connectance. Comparing the results here to those from a previous study with tebufenozide, this was selectively toxic to cladoceran and had tittles effect on food web stability, indicates that differential sensitivity among taxa can influences the ecological significance of pesticides effects on Zooplankton communities (Kreutzweiser et al., 2004).

Cautions

It is invariably believed that medicines or pesticides of plant origin are safe and can be used without any precaution. But it is not so. Sometimes they have serious side effects. Hence, medicines of plant origin should be treated with the some caution as medicines of synthetic origin. Neem oil seems to be particular concern. Its consumption, although widely practiced in different parts of Asia, is not recommended. The toxicological nature of neem is also harmful for the pregnant women. A higher dose can cause mortality. The leaves or leaf extracts also should not be consumed by people or fed to animals over a long period. There are reports of renal failure in Ghanaians who were drinking leaf teas as malarial treatment. Each preparation needs detailed toxicological evaluation before its commercial use.

Conclusion

Neem tree can be regarded as a valuable tree for rationalization of its use in traditional preparations of modern drugs and pesticides development. Though detailed pharmacokinetical/ toxicokinetical data on the constituents are not available, possible modes of action are interference with hormonal regulation, inhibition of various enzymes, interaction with receptors and alteration of membrane permeability and integrity. In general, the toxicity of leaf and bark extracts and isolated limonoides is very low. However, the seed oil is toxic and hence its use in large amounts may prove hazardous (Nat Vander et al., 1991).

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